PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

DANIELS, Jeffrey, Nicholas
Page White & Farrer
54 Doughty Street
London WC1N 2LS
ROYAUME-UNI

Date of mailing (day/month/year)
25 September 2000 (25.09.00)

Applicant's or agent's file reference
100794/JD/JE

IMPORTANT NOTIFICATION

International application No.
PCT/GB99/03830

International filing date (day/month/year)
17 November 1999 (17.11.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
CAMBRIDGE MOLECULAR TECHNOLOGIES LIMITED
Unit 3
Cambridge Techno Park
Newmarket Road
Cambridge CB5 8PB
United Kingdom

State of Nationality: GB
State of Residence: GB
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
CAMBRIDGE MOLECULAR TECHNOLOGIES LIMITED
Granta Park
Abington
Cambridge CB1 6GR
United Kingdom

State of Nationality: GB
State of Residence: GB
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
Catherine Massetti

Telephone No.: (41-22) 338.83.38

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PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

HALLYBONE, Huw, George
Carpmaels & Ransford
43 Bloomsbury Square
London WC1A 2RA
ROYAUME-UNI

Date of mailing (day/month/year) 18 April 2001 (18.04.01)	
Applicant's or agent's file reference 100794/JD/JE	**IMPORTANT NOTIFICATION**
International application No. PCT/GB99/03830	**International filing date** (day/month/year) 17 November 1999 (17.11.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
CAMBRIDGE MOLECULAR TECHNOLOGIES LIMITED Granta Park Abington Cambridge CB1 6GR United Kingdom	GB	GB
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
WHATMAN BIOSCIENCE LIMITED Granta Park Abington Cambridge CB1 6GR United Kingdom	GB	GB
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer R. Chrem
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994) 003969298

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

HALLYBONE, Huw, George
Carpmaels & Ransford
43 Bloomsbury Square
London WC1A 2RA
ROYAUME-UNI

Date of mailing (day/month/year) 16 January 2001 (16.01.01)	
Applicant's or agent's file reference 100794/JD/JE	**IMPORTANT NOTIFICATION**
International application No. PCT/GB99/03830	**International filing date** (day/month/year) 17 November 1999 (17.11.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
CAMBRIDGE MOLECULAR TECHNOLOGIES LIMITED Granta Park Abington Cambridge CB1 6GR United Kingdom	GB	GB
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
WHATMAN BIOSCIENCE LIMITED Granta Park Abington Cambridge CB1 6GR United Kingdom	GB	GB
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer R. Chrem
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 20 July 2000 (20.07.00)	
International application No. PCT/GB99/03830	**Applicant's or agent's file reference** 100794/JD/JE
International filing date (day/month/year) 17 November 1999 (17.11.99)	**Priority date** (day/month/year) 17 November 1998 (17.11.98)
Applicant BUTT, Neil, James et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

06 June 2000 (06.06.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Zakaria EL KHODARY

Telephone No.: (41-22) 338.83.38

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For Receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 100794/JD/JE

Box No. I TITLE OF INVENTION

ISOLATING NUCLEIC ACID

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Cambridge Molecular Technologies Limited
Unit 3 Cambridge Techno Park
Newmarket Road
Cambridge CB5 8PB
United Kingdom

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

BUTT Neil James
15 Petworth Street
Cambridge
CB2 2LY
United Kingdom

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

DANIELS, Jeffrey Nicholas
Page White & Farrer
54 Doughty Street
London WC1N 2LS
United Kingdom

Telephone No. 0171 831 7929

Facsimile No. 0171 831 8040

Teleprinter No. 8955681

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

JONES Christopher Peter
84 Fowlmere Road
Heydon
Herts
SG8 8PU
United Kingdom

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

---

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

---

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

---

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

---

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- [x] AP **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SL Sierra Leone, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- [x] EA **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- [x] EP **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- [x] OA **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- [x] AE United Arab Emirates
- [x] AL Albania .....
- [x] AM Armenia .....
- [x] AT Austria .....
- [x] AU Australia .....
- [x] AZ Azerbaijan
- [x] BA Bosnia and Herzegovina .....
- [x] BB Barbados
- [x] BG Bulgaria .....
- [x] BR Brazil .....
- [x] BY Belarus .....
- [x] CA Canada
- [x] CH and LI Switzerland and Liechtenstein
- [x] CN China .....
- [x] CU Cuba .....
- [x] CZ Czech Republic .....
- [x] DE Germany .....
- [x] DK Denmark .....
- [x] EE Estonia .....
- [x] ES Spain .....
- [x] FI Finland .....
- [x] GB United Kingdom
- [x] GD Grenada
- [x] GE Georgia .....
- [x] GH Ghana .....
- [x] GM Gambia
- [x] HR Croatia .....
- [x] HU Hungary .....
- [x] ID Indonesia
- [x] IL Israel .....
- [x] IN India .....
- [x] IS Iceland
- [x] JP Japan .....
- [x] KE Kenya .....
- [x] KG Kyrgyzstan .....
- [x] KP Democratic People's Republic of Korea .....
- [x] KR Republic of Korea .....
- [x] KZ Kazakhstan .....
- [x] LC Saint Lucia
- [x] LK Sri Lanka
- [x] LR Liberia
- [x] LS Lesotho .....
- [x] LT Lithuania
- [x] LU Luxembourg
- [x] LV Latvia
- [x] MD Republic of Moldova .....
- [x] MG Madagascar .....
- [x] MK The former Yugoslav Republic of Macedonia .....
- [x] MN Mongolia
- [x] MW Malawi .....
- [x] MX Mexico .....
- [x] NO Norway
- [x] NZ New Zealand .....
- [x] PL Poland .....
- [x] PT Portugal .....
- [x] RO Romania
- [x] RU Russian Federation .....
- [x] SD Sudan
- [x] SE Sweden
- [x] SG Singapore
- [x] SI Slovenia .....
- [x] SK Slovakia .....
- [x] SL Sierra Leone .....
- [x] TJ Tajikistan .....
- [x] TM Turkmenistan .....
- [x] TR Turkey .....
- [x] TT Trinidad and Tobago .....
- [x] UA Ukraine .....
- [x] UG Uganda .....
- [x] US United States of America .....
- [x] UZ Uzbekistan .....
- [x] VN Viet Nam .....
- [x] YU Yugoslavia .....
- [x] ZA South Africa .....
- [x] ZW Zimbabwe .....

Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet:

- [x] Tanzania .....
- [x] Morocco .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Supplemental Box *If the Supplemental Box is not used, this sheet should not be included in the request.*

1. If, in any of the Boxes, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

(i) if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;

(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;

(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;

(iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;

(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "continuation" or "continuation-in-part": in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;

(vi) if, in Box No. VI, there are more than three earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;

(vii) if, in Box No. VI, the earlier application is an ARIPO application: in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.

2. If, with regard to the precautionary designation statement contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.

3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty: in such case, write "Statement concerning non-prejudicial disclosures or exceptions to lack of novelty" and furnish that statement below.

CONTINUATION OF BOX IV

Agents continued

PENDLEBURY, DR. ANTHONY (GB)
PALMER, ROGER (GB)
DRIVER, VIRGINIA ROZANNE (GB)
JENKINS, PETER DAVID (GB)
RICHARDS, DAVID JOHN (GB)
STYLE, KELDA CAMILLA KAREN (GB)
NEOBARD, WILLIAM JOHN (GB)
SHACKLETON, NICOLA (GB)
SLINGSBY, PHILIP ROY (GB)
HILL, DR. CHRISTOPHER MICHAEL (GB)
RUUSKANEN, JUHA-PEKKA (FI)

of PAGE WHITE & FARRER, 54 DOUGHTY STREET, LONDON WC1N 2LS, GB

Tel: 0171 831-7929 Fax: 0171 831-8040 Telex: 8955681

Box No. VI PRIORITY CL[redacted] ☐ Further priority [redacted] are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 17/11/98	9825215.8	U.K.		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): (1)

** Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)* : ISA/ EP

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*: Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following number of sheets:

request	:	5
description (excluding sequence listing part)	:	8
claims	:	5
abstract	:	1
drawings	:	–
sequence listing part of description	:	–
Total number of sheets	:	19

This international application is accompanied by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: Form 23/77

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: GB

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

DANIELS, Jeffrey Nicholas
(Authorised Representative)

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA/
6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ EP

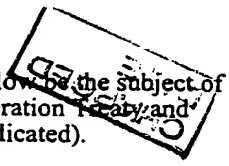
PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:

The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).



For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference:

International application No.	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/GB99/03830	17.11.1999	17.11.1998

Title of invention

ISOLATING NUCLEIC ACID

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Cambridge Molecular Technologies Limited
Granta Park
Abington
Cambridge CB1 6GR
United Kingdom

Telephone No.:

Facsimile No.:

Teleprinter No.:

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
GB	GB

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

BUTT, Neil James
15 Petworth Street
Cambridge
CB2 2LY
United Kingdom

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
GB	GB

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

JONES, Christopher Peter
84 Fowlmere Road
Heydon
Herts
SG8 8PU
United Kingdom

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
GB	GB

☐ Further applicants are indicated on a continuation sheet.

International application No.
PCT/GB99/03830

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [X] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

DANIELS, Jeffrey Nicholas
Page White & Farrer
54 Doughty Street
London WC1N 2LS
United Kingdom

Telephone No.: 020 7831 7929

Facsimile No.: 020 7831 8040

Teleprinter No.: 8955681

[ ] **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special addr ess to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination **to start on the basis of:**

[X] the international application as originally filed

the description [ ] as originally filed
[ ] as amended under Article 34

the claims [ ] as originally filed
[ ] as amended under Article 19 (together with any accompanying statement)
[ ] as amended under Article 34

the drawings [ ] as originally filed
[ ] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [ ] The applicant wishes the start of the international preliminary examination **to be postponed** until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

[X] which is the language in which the international application was filed.

[ ] which is the language of a translation furnished for the purposes of international search.

[ ] which is the language of publication of the international application.

[ ] which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby **elects all eligible States** *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes **not to elect:**

Sheet No. 3.

International application No.
PCT/GB99/03830

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:				For International Preliminary Examining Authority use only: received	For International Preliminary Examining Authority use only: not received
1. translation of international application	:		sheets	☐	☐
2. amendments under Article 34	:		sheets	☐	☐
3. copy (or, where required, translation) of amendments under Article 19	:		sheets	☐	☐
4. copy (or, where required, translation) of statement under Article 19	:		sheets	☐	☐
5. letter	:	1	sheets	☐	☐
6. other *(specify)*	:		sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. [X] fee calculation sheet
2. [ ] separate signed power of attorney
3. [ ] copy of general power of attorney; reference number, if any:
4. [ ] statement explaining lack of signature
5. [ ] nucleotide and or amino acid sequence listing in computer readable form
6. [ ] other *(specify)*:

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

.....
DANIELS, Jeffrey Nicholas
(Authorised Representative)

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

DUE: 19-12-00

PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

DANIELS,JEFFREY N.
PAGE WHITE & FARRER
54 Doughty Street
LONDON WC1N 2LS
GRANDE BRETAGNE

RECEIVED
25 SEP 2000
Ans'a.....

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	19.09.2000
Applicant's or agent's file reference: 100794/JD/JE	**REPLY DUE** **within 3 month(s)** from the above date of mailing
International application No.: PCT/GB99/03830	International filing date *(day/month/year)*: 17/11/1999
Priority date *(day/month/year)*: 17/11/1998	
International Patent Classification (IPC) or both national classification and IPC: C12N15/10	
Applicant: CAMBRIDGE MOLECULAR TECHNOLOGIES LIMITED	

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☒ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain document cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

   When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

   How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

   Also: For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis. For an informal communication with the examiner, see Rule 66.6.

   If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 17/03/2001.

Name and mailing address of the international preliminary examining authority:
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer / Examiner
Mundel, C

Formalities officer (incl. extension of time limits)
Vullo, C
Telephone No. +49 89 2399 8061

I. Basis of th opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".)*:

Description, pages:

1-8 as originally filed

Claims, No.:

1-41 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

II. Priority

1. ☐ This opinion has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

s e s parat sheet

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	27-28 and 31 (NO)
Inventive step (IS)	Claims	
Industrial applicability (IA)	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item II
Priority

The priority document of the present application was not available at the time where this preliminary opinion has been drafted. The present analysis is based on the hypothesis that all the claims have a priority right corresponding to the date of filing of the priority document (17.11.98).

Re Item V
Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present application refers to methods for isolating plasmid DNA from a DNA containing material which comprises plasmid DNA and genomic DNA. In said methods, the plasmid DNA is extracted into a water-immiscible organic solvent by mixing the material with the organic solvent, a chaotrope and water under conditions to denature the genomic DNA. The application also refers to extraction mixtures for use in said methods.

2. **Lack of novelty and inventive step; articles 33(2) and 33(3) PCT.**

   Due to the clarity problem mentioned in point VIII-11 of the present opinion, the subject-matter of claims 27-28 and 31 refers to a mixture comprising inter alia a water-immiscible organic solvent, a chaotrope and water (and optionally a base like sodium hydroxide) without any consideration of the concentration of the different compounds. The IPEA is the opinion that such mixtures have already been disclosed in the art and, therefore, claims 27-28 and 31 can not be considered as new or inventive.

Re Item VIII
Certain observations on the international application

Lack of clarity; article 6 PCT.

1. As a general remark, the attention of the applicant is drawn to the fact that the use of the term "comprises" in numerous claims of the present application renders the wording of said claims confuse since it implies that the scope of the claim is not limited to the particular compound cited but could also include different other not mentioned compounds.
   This remark applies in particular to claims 5-7, 9-11, 20-23, 26, 28-30 and 32-34.

2. Claim 1 of the present application lacks clarity for the following reasons :
   (i) The water-immiscible solvent is only characterized by the fact that it is capable of supporting plasmid DNA, i.e. by the result to be achieved. According to the PCT Gazette of the 29.10.98 "PCT International Preliminary Examination Guidelines", Chapter III-4.7 : "The area defined by the claims must be as precise as the invention allows. As a general rule, claims which attempt to define the invention, or a feature thereof, by a result to be achieved should be objected to".
   This remark also applies to independent claim 27 but concerning the extraction mixture and to most of the depending claims.
   (ii) The conditions for the extraction step (i) are characterized by the result to be achieved, i.e. "conditions to denature the genomic DNA", what should be avoided (see point VIII-2 (i) above).
   (iii) The wording of claim 1 implies that the method can be used on bacterial culture but also on any eucaryotic cell containing plasmid DNA. However, the description of the present application only discloses the use of the method on a bacterial culture. The IPEA is the opinion that, due to the differences in the organisation of prokaryotic and eucaryotic cells, the methods of the present application can not be generalized to eucaryotic cells and, therefore, considers that the use of said methods on eucaryotic cells is not supported by the description (article 5 PCT).

3. In claim 2, the organic solvent is characterized by the fact that it "is capable of selectively supporting the plasmid DNA to the exclusion of denatured genomic DNA", i.e by the result to be achieved what should be avoided (see point VIII-2). This remark also applies to claim 31 but concerning the extraction mixture and to most of the depending claims.

4. Claim 3 refers to a method wherein the conditions to denature the plasmid DNA comprise, inter alia, basic conditions. This claim is unclear for the following reasons :
   (i) There is no clear definition of what the basic conditions should be. A pH of the mixture of 7.5 - which is considered as a basic pH - will probably not be sufficient for the denaturation of DNA.
   (ii) Claim 1 refers to the fact that the extracting step (i) should happen under conditions to denature the **genomic** DNA. Claim 3 which is dependent of claim 1 define conditions to denature the **plasmid** DNA. It is, therefore, not clear if it is the plasmid DNA or the genomic DNA or both which should be denatured. This remark is also valid for claim 4.

5. Claim 4 refers to the method as claimed in claim 3 wherein the conditions to denature the plasmid DNA comprise basic conditions in which a base is present. Claim 4 appears to be redundant with claim 3 since the basic conditions disclosed in the application always imply the presence of a base.

6. Claim 5 refers to a method as claimed in claim 4 wherein the organic solvent comprises a C_3 to C_6 alcohol. This claim lacks clarity for the following reasons :
   (i) The use of the term "**comprises**" implies that the organic solvent could also comprise lots of other compounds. There is no example in the description of the present application of what such additional compounds could be and this other compounds would not, therefore, be considered as supported by the description (article 5 PCT).
   (ii) The attention of the applicant is drawn to the fact that the only organic solvents which have been used in the present application are C_4 alcohols and more particularly N-Butanol, 2 methyl propanol and Butan-2-ol. Therefore, the use of C_3, C_5 and C_6 alcohols and C_4 alcohols other than N-Butanol, 2 methyl propanol and Butan-2-ol can not be considered as

supported by the description of the present application (article 5 PCT). Moreover, some of the alcohols encompassed by claim 5 may not have the property to be water-immiscible, like for example isopropanol which is a C_3 alcohol and which was found not to be water-immiscible (p.7 of the present application).

7. Claim 13 refers to the method of claim 12 wherein the amount of organic solvent is in the range from 20% to 70% based on the volume of the combination of organic solvent, chaotrope and water (i.e. : the extraction mixture). The attention of the applicant is drawn to the fact that, even if the concentration of organic solvent is defined in the extraction mixture, the concentration of said solvent in the mixture [extraction mixture + plasmid containing material] could be very low since no ratio [extraction mixture/plasmid containing material] is given. The IPEA is the opinion that the scope of claim 13 would be clearer if the ratio [extraction mixture / plasmid containing material] would be defined or if the concentration of the organic solvent would be given by reference to the final volume of the mixture [extraction mixture + plasmid containing material].
This remark also applies to claims 14-15 and to claims 16-17 but concerning the concentration of the chaotrope.

8. In claim 18, the precipitating agent is only characterized by the fact that it "can precipitate the plasmid DNA from the organic solvent", i.e. by the result to be achieved what should be avoided (see point VIII-2).

9. Claim 20 of the present application refers to the use of alcohols as precipitating agents. The attention of the applicant is drawn to the fact that the description of the present application only discloses the use of ethanol as a precipitating agent. Therefore, the use of other alcohols as precipitating agents could be considered as not supported by the description (article 5 PCT).
Moreover, the attention of the applicant is drawn to the fact that all the alcohols can not be used as precipitating agents. For example, n-butanol, 2 methyl propanol and butan-2-ol - which are alcohols - are used as water-immiscible solvent for the extraction of plasmid DNA in the present application and should, therefore, not be able to precipitate plasmid DNA.

10. Claim 24 refers to "the method as claimed in any one of the preceding claims, which further comprises the step of separating the organic and aqueous phases of step (i) prior to recovering the plasmid DNA". The IPEA is the opinion that the subject-matter of this claim is redundant with the subject-matter of claim 1 since the separation of the organic and aqueous phases will always occur, the organic solvent being water-immiscible.

11. Claim 27 refers to an extraction mixture for selectively extracting plasmid DNA from a DNA-containing material, which extraction mixture comprises a water-immiscible organic solvent capable of supporting plasmid DNA, a chaotrope and water.
Considering the facts that :
- According to the PCT Gazette of the 29.10.98 "PCT International Preliminary Examination Guidelines", Chapter IV-7.6 : in interpreting claims for the consideration of novelty, the examiner "should remember that non-distinctive characteristics of a particular intended use should be disregarded".
- The water immiscible organic solvent can not be defined by the fact that it is "capable of supporting plasmid DNA" (see point VIII-2 (i)).
- The wording "extraction mixture" has no real technical meaning.
- There is no concentration given for the different compounds cited.
The IPEA considers that claim 27 can be seen as a mixture comprising **inter alia** : a water-immiscible solvent, a chaotrope and water without any consideration of concentration of the different components. The IPEA is the opinion that such mixtures are already known in the art, even if not used for the extraction of plasmid DNA, and would therefore deprive claim 27 of novelty.
This remark also applies to claims 28 and 31.
This remark also applies to a lesser extent to most of the other dependent claims.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:
DANIELS, JEFFREY N.
PAGE WHITE & FARRER
54 Doughty Street
LONDON WC1N 2LS
GRANDE BRETAGNE

RECEIVED
- 3 JUL 2000

NOTIFICATION OF RECEIPT OF DEMAND BY COMPETENT INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

(PCT Rules 59.3(e) and 61.1(b), first sentence and Administrative Instructions, Section 601(a))

Date of mailing *(day/month/year)*	30.06.00
Applicant's or agent's file reference: 100794/JD/JE	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB 99/03830	17/11/1999	17/11/1998

Applicant

CAMBRIDGE MOLECULAR TECHNOLOGIES LIMITED et al.

1. The applicant is hereby **notified** that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

06/06/2000

2. This date of receipt is:

- [X] the actual date of receipt of the demand by this Authority (Rule 61.1(b)).
- [ ] the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).
- [ ] the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. [ ] **ATTENTION:** That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide*, Volume II.

[ ] *(If applicable)* This notification confirms the information given by telephone, facsimile transmission or in person on:

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/
European Patent Office
D-80298 Munich
Tel. (+49-89) 2399-0, Tx: 523656 epmu d
Fax: (+49-89) 2399-4465

Authorized officer
PITARD J A S
Tel. (+49-89) 2399-2156

EUROPÄISCHES PATENTAMT · EUROPEAN PATENT OFFICE · OFFICE EUROPÉEN DES BREVETS

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

DANIELS, Jeffrey, Nicholas
Page White & Farrer
54 Doughty Street
London WC1N 2LS
ROYAUME-UNI

RECEIVED
24 DEC 1999

Date of mailing (day/month/year) 14 December 1999 (14.12.99)	
Applicant's or agent's file reference 100794/JD/JE	**IMPORTANT NOTIFICATION**
International application No. PCT/GB99/03830	International filing date (day/month/year) 17 November 1999 (17.11.99)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 17 November 1998 (17.11.98)
Applicant CAMBRIDGE MOLECULAR TECHNOLOGIES LIMITED et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
17 Nove 1998 (17.11.98)	9825215.8	GB	06 Dece 1999 (06.12.99)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

Taïeb Akremi

Telephone No. (41-22) 338.83.38

WO 00/29563
PCT/GB99/03830

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
DANIELS, Jeffrey, Nicholas
Page White & Farrer
54 Doughty Street
London WC1N 2LS
ROYAUME-UNI

Date of mailing (day/month/year)	
25 May 2000 (25.05.00)	
Applicant's or agent's file reference 100794/JD/JE	**IMPORTANT NOTICE**

International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/GB99/03830	17 November 1999 (17.11.99)	17 November 1998 (17.11.98)

Applicant
CAMBRIDGE MOLECULAR TECHNOLOGIES LIMITED et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,CN,JP,KP,KR,MA,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CU,CZ,DE,DK,EA,EE,EP,ES,FI,GB,GD,GE,GH,GM,
   HR,HU,ID,IL,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NO,NZ,OA,PL,PT,
   RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   25 May 2000 (25.05.00) under No. WO 00/29563

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a demand for international preliminary examination must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the national phase, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

Th International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

09/856336

Rec'd PCT/PTO 17 MAY 2001

CARPMAELS & RANSFORD

CHARTERED PATENT ATTORNEYS EUROPEAN PATENT ATTORNEYS TRADE MARK ATTORNEYS

TELEPHONE 020-7242 8692
FACSIMILE 020-7405 4166
020-7831 8501
WEBSITE www.carpmaels.com
E-MAIL email@carpmaels.com

43 BLOOMSBURY SQUARE
LONDON WC1A 2RA

AND AT MUNICH

European Patent Office
Erhardtstrasse 27
D-80298 Munich
GERMANY

YOUR REF

OUR REF P025849WO/hgh/cjm

19th December 2000

Dear Sirs,

Re: International patent application PCT/GB99/03830
Cambridge Molecular Technologies Limited

Representation of the above-mentioned patent application has been transferred to this firm. Appointment of agent forms signed by the applicants are enclosed. You will note that one of the forms has been signed on behalf of WHATMAN BIOSCIENCE LIMITED. Since the filing date, CAMBRIDGE MOLECULAR TECHNOLOGIES LIMITED has changed its name to WHATMAN BIOSCIENCE LIMITED, and this change is currently being recorded by the International Bureau at WIPO. A copy of the change of name certificate is enclosed for your information.

In response to the written opinion issued on 19th September 2000, please substitute the enclosed amended claims for those currently on file. The deletion of subject-matter from the claims does not indicate that is has been abandoned, and the right to re-instate deleted subject-matter or to file divisional or continuation applications in the future is specifically reserved.

FACSIMILE MESSAGE

To: **EPO Munich**
Fax No.: **00 49 89 2399 4465**

This fax comprises 17 sheets. If a sheet is missing, or imperfectly received, please contact us immediately (Tel: 020-7242 8692; Fax: 020-7405 4166). If you are not the addressee, please contact us immediately and then destroy this fax.

The amended claims have been restricted to specify that the "water-immiscible organic solvent capable of supporting plasmid DNA" is butanol. This limitation finds basis in claim 6 as filed.

The claim amendments filed herewith deal fully with the objections raised in sections 1, 2(i), 3, 4 and 5 of item VIII of the written opinion.

In section 2(ii), the examiner has argued that the phrase "conditions to denature the genomic DNA" defines the invention by a result to be achieved. Whilst this is true, the result to be achieved (*i.e.* DNA denaturation) is trivial. Many ways are known for denaturing DNA (*e.g.* heating, use of alkali conditions *etc.* – see Sambrook *et al. Molecular Cloning: a Laboratory Manual*, 2nd edition (1989) page 1.22), and the precise way in which the genomic DNA is denatured is irrelevant to the way the invention functions. Moreover, in the circumstances specified in the claims (*i.e.* in the presence of butanol and a chaotrope) denaturation is essentially inherent.

Similar objections are raised in sections 8 and 9. Methods for precipitating DNA from organic solvents (*e.g.* using alcohols) are trivial and well-known (*e.g.* see Sambrook *et al.*, pages E.10-E.15). The requirements of claim 18 as filed (now claim 15) do not place an undue burden on the skilled person.

Put another way, it would be unfair to require the claims to be restricted to the use of a particular denaturant or precipitating agent – the invention in its broadest sense lies in the generic finding that plasmid and genomic DNA partition differently in particular aqueous/organic solvent mixtures, and the claims should be similarly generic.

In section 2(iii), the examiner has argued that the claimed method cannot be applied to eukaryotic cells. This objection is wholly unsupported – no reasoned or rational technical basis has been given and, in particular, no *a priori* reason for expecting failure has been provided. The invention relates to DNA extraction and, whilst its cellular arrangement may differ, the DNA of prokaryotes and eukaryotes is the same chemical compound and can be expected to behave in the same way in the solvent systems defined in the claims. The examiner has not raised "serious doubts substantiated by verifiable facts" (T19/90), and the objection should be withdrawn.

Section 6(ii) has largely been dealt with by the claim amendments. The examiner appears, however, to be objecting to the breadth of the term "butanol". In particular, it has been stated that "C_4 alcohols other than N-Butanol, 2 methyl propanol and Butan-2-ol cannot be considered as supported". The reference to other "alcohols" is not understood, as there are only four structural forms of butanol (*i.e.* C_4 alcohol), but it is

submitted that it is reasonable to extrapolate the data provided for 3 of the 4 forms. There is thus adequate support for all forms of butanol.

Section 7 does not raise an objection, but merely states that the examiner thinks that the claim could be "clearer". As long as a claim is clear, however, the requirements of Article 6 PCT are satisfied, regardless of whether "clearer" forms could be envisaged. Even so, the examiner should note that the method will typically involve adding solid material to a large volume of solvent mixture. The volume of the "plasmid containing material" will thus be negligible. Ample guidance in this respect is provided by the description.

The objection raised in section 10 is incorrect, as separation of the two phases from each other is not necessarily a feature of the invention. It may be, however, that the examiner has mis-understood the meaning of English word "separating" as used in the claim. Whilst two immiscible solvents will be "separated" in the sense that they are not mixed, the claim refers to the physical separation of the two solvents *e.g.* removing the upper phase into a pipette.

In section 11, the examiner has argued that the claimed composition "are already known in the art", but has not provided any evidence of this. It is completely inappropriate to raise lack of novelty objections in this way. The examiner must either provide a document showing that the claimed compositions were made available to the public before the relevant date, or should withdraw this objection.

If the Examiner does not agree with the arguments set forth above and is minded to issue an unfavourable IPER, a further opportunity to submit arguments or amendments is requested [Rule 66.4(b) PCT] and the option of a telephone discussion with the Examiner is requested [Article 34(2)(a) PCT].

Yours truly,

HALLYBONE, Huw George

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VCJM

PATENT COOPERATION TREATY

P02584900

PCT

From the INTERNATIONAL BUREAU

To:

HALLYBONE, Huw, George
Carpmaels & Ransford
43 Bloomsbury Square
London WC1A 2RA
ROYAUME-UNI

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

Date of mailing (day/month/year)
18 April 2001 (18.04.01)

Applicant's or agent's file reference
100794/JD/JE

IMPORTANT NOTIFICATION

International application No.
PCT/GB99/03830

International filing date (day/month/year)
17 November 1999 (17.11.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
CAMBRIDGE MOLECULAR TECHNOLOGIES LIMITED
Granta Park
Abington
Cambridge CB1 6GR
United Kingdom

State of Nationality: GB
State of Residence: GB
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address
WHATMAN BIOSCIENCE LIMITED
Granta Park
Abington
Cambridge CB1 6GR
United Kingdom

State of Nationality: GB
State of Residence: GB
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Gen va 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
R. Chrem
Telephone No.: (41-22) 338.83.38

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
100794/JD/JE		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/03830	17/11/1999	17/11/1998
International Patent Classification (IPC) or national classification and IPC C12N15/10		
Applicant CAMBRIDGE MOLECULAR TECHNOLOGIES LIMITED		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06/06/2000	14.02.01
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Mundel, C Telephone No. +49 89 2399 7314

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/03830

I. Basis f th report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-8	as originally filed				
Claims, No.:					
1-35	as received on	19/12/2000	with letter of	19/12/2000	

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:
   - ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

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(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

   Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
   see separate sheet

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-35
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-35
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-35
	No:	Claims	

2. Citations and explanations
   see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

R It m II
Priority

The priority document of the present application was not available at the time where this International Preliminary Examination Report (IPER) has been drafted. The present analysis is based on the hypothesis that all the claims have a priority right corresponding to the date of filing of the priority document (17.11.98).

Re Item V
Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present application refers to methods for isolating plasmid DNA from a DNA containing material which comprises plasmid DNA and genomic DNA. In said methods, the plasmid DNA is extracted into butanol by mixing the material with the butanol, a chaotrope and water under conditions to denature the genomic DNA. The application also refers to extraction mixtures for use in said methods.

2. The new claims 1-35 filed with the letter of 19.12.00 are allowable under articles 19(2) and 34(2)(b) PCT.

3. The arguments of the applicant filed with the letter of 19.12.00 have been taken into account for drafting the present IPER.

4. **Novelty and inventive step; articles 33(2) and 33(3) PCT.**

   The subject-matter of the present claims has never been disclosed or suggested in the documents cited in the International Search Report (ISR). Therefore, claims 1-35 are considered as new and inventive in the sense of articles 33(2) and 33(3) PCT.

R It m VIII

Certain observations on the international application

Lack of clarity; article 6 PCT.

1. Claim 15 of the present application refers to the use of a precipitating agent in the method of any claim 1-14. Said agents are only characterized by the fact that they "can precipitate the plasmid DNA from butanol", i.e. by the result to be achieved by said precipitating agents.
According to the PCT Gazette of the 29.10.98 "PCT International Preliminary Examination Guidelines", Chapter III-4.7 : "The area defined by the claims must be as precise as the invention allows. As a general rule, claims which attempt to define the invention, or a feature thereof, by a result to be achieved should be objected to".
The attention of the applicant is drawn to the fact that even if precipitating agents for precipitating plasmid DNA in an aqueous solution are well-known in the art, it is not obvious that said agents, with the exception of ethanol used in the examples of the present application, will also be efficient to precipitate DNA in butanol. Therefore, the use of agents other than ethanol for precipitating plasmid DNA from butanol can not be considered as supported by the description of the present application (article 5 PCT in combination with article 6 PCT).
This remark is also valid for claim 17.

2. Claim 17 refer to the use of general alcohols as precipitating agents. The attention of the applicant is drawn to the fact that not all the alcohols can be used for precipitating the DNA from the butanol (see also point VIII-1 above). For example, n-butanol, 2 methyl propanol and butan-2-ol - which are alcohols - are used as water-immiscible solvent for the extraction of plasmid DNA in the present application and should, therefore, not be able to precipitate plasmid DNA.

CLAIMS:

1. A method for isolating plasmid DNA from a DNA containing material which comprises plasmid DNA and genomic DNA, comprising:

(i) extracting the plasmid DNA into a water-immiscible organic solvent capable of supporting plasmid DNA, by mixing the material with the organic solvent, a chaotrope and water under conditions to denature the genomic DNA; and

(ii) recovering the plasmid DNA from the organic phase.

2. A method as claimed in claim 1, wherein the organic solvent is capable of selectively supporting the plasmid DNA to the exclusion of denatured genomic DNA.

3. A method as claimed in claim 1 or claim 2, wherein the conditions to denature the plasmid DNA comprise basic conditions or a temperature of at least 65°C.

4. The method as claimed in claim 3, wherein the conditions to denature the plasmid DNA comprise basic conditions in which a base is present.

5. A method as claimed in claim 4, wherein the organic solvent comprises a C_3 to C_6 alcohol.

6. A method as claimed in claim 5, wherein the C_3 to C_6 alcohol comprises butanol.

7. A method as claimed in claim 6, wherein the butanol comprises n-butanol.

8. A method as claimed in any one of claims 4 to 7, wherein the chaotrope is selected from the group consisting of guanidine

hydrochloride, guanidine thiocyanate, sodium perchlorate and mixtures thereof.

9. A method as claimed in claim 8, wherein the chaotrope comprises guanidine hydrochloride.

10. A method as claimed in any one of claims 4 to 9, wherein the base comprises a hydroxide.

11. A method as claimed in claim 10, wherein the hydroxide comprises sodium hydroxide.

12. A method as claimed in any one of claims 4 to 11, wherein the organic solvent, the chaotrope, the base and the water are combined to form an extraction mixture, and extraction step (i) comprises mixing the extraction mixture with the plasmid DNA-containing material.

13. A method as claimed in any one of claims 4 to 12, wherein the amount of organic solvent is in the range from 20 to 70% based on the volume of the combination of organic solvent, chaotrope and water.

14. A method as claimed in claim 13, wherein the amount of the organic solvent is in the range from 35 to 50%.

15. A method as claimed in claim 14, wherein the amount of the organic solvent is about 42%.

16. A method as claimed in any one of claims 4 to 15, wherein the chaotrope is present at a concentration of from 0.7M to 1.2M based on the combination of organic solvent, chaotrope and water.

17. A method as claimed in claim 16, wherein the concentration of the chaotrope is about 0.9M.

18. A method as claimed in any one of claims 4 to 17, wherein the recovery step (ii) comprises mixing the DNA-containing organic phase with a precipitating agent that can precipitate the plasmid DNA from the organic solvent, and separating the precipitated plasmid DNA from the solvent.

19. A method as claimed in claim 18, wherein the recovery step (ii) further comprises a washing step in which the precipitated plasmid DNA is washed.

20. A method as claimed in claim 18 or claim 19, wherein the precipitating agent comprises an alcohol.

21. A method as claimed in claim 20, wherein the alcohol comprises ethanol.

22. A method as claimed in any one of claims 18 to 21, wherein the precipitating agent further comprises an acetate salt.

23. A method as claimed in claim 22, wherein the acetate salt comprises sodium acetate.

24. A method as claimed in any one of the preceding claims, which further comprises a step of separating the organic and aqueous phases of step (i) prior to recovering the plasmid DNA.

25. A method as claimed in claim 24, wherein the step of separating the organic and aqueous phases further comprises centrifugation of the mixture formed in step (i) to facilitate separation of the mixture into the organic and aqueous phases.

26. A method as claimed in any one of the preceding claims, wherein the DNA-containing material comprises a lysed or unlysed bacterial culture.

27. An extraction mixture for selectively extracting plasmid DNA from a DNA-containing material, which extraction mixture comprises a water-immiscible organic solvent capable of supporting plasmid DNA, a chaotrope and water.

28. An extraction mixture as claimed in claim 27, which further comprises a base.

29. An extraction mixture as claimed in claim 28, wherein the base comprises a hydroxide.

30. An extraction mixture as claimed in claim 29, wherein the hydroxide comprises sodium hydroxide.

31. An extraction mixture as claimed in any one of claims 27 to 30, wherein the organic solvent is capable of selectively supporting plasmid DNA to the exclusion of genomic DNA.

32. An extraction mixture as claimed in any one of claims 27 to 31, wherein the organic solvent comprises a C_3 to C_6 alcohol.

33. An extraction mixture as claimed in any one of claims 27 to 32, wherein the organic solvent comprises butanol.

34. An extraction mixture as claimed in claim 33, wherein the butanol comprises n-butanol.

35. An extraction mixture as claimed in any one of claims 27 to 33, wherein the organic solvent constitutes from 20 to 70% based on the volume of the extraction mixture.

36. An extraction mixture as claimed in claim 35, wherein the organic solvent constitutes from 35 to 50 % of the extraction mixture.

37. An extraction mixture as claimed in claim 36, wherein the organic solvent constitutes about 42% of the extraction mixture.

38. An extraction mixture as claimed in any one of claims 27 to 37, wherein the chaotrope is selected from the group consisting of guanidine hydrochloride, guanidine thiocyanate, sodium perchlorate and mixtures thereof.

39. An extraction mixture as claimed in claim 38, wherein the chaotrope comprises guanidine hydrochloride.

40. An extraction mixture as claimed in any one of claims 27 to 39, wherein the concentration of chaotrope in the extraction mixture is from 0.7M to 1.2M.

41. An extraction mixture as claimed in claim 40, wherein the concentration of the chaotrope in the extraction mixture is about 0.9M.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(71) Applicant *(for all designated States except US)*: **CAMBRIDGE MOLECULAR TECHNOLOGIES LIMITED** [GB/GB]; Unit 3, Cambridge Techno Park, Newmarket Road, Cambridge CB5 8PB (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BUTT, Neil, James** [GB/GB]; 15 Petworth Street, Cambridge CB2 2LY (GB). **JONES, Christopher, Peter** [GB/GB]; 84 Fowlmere Road, Heydon, Herts SG8 8PU (GB).

(74) Agents: **DANIELS, Jeffrey, Nicholas** et al.; Page White & Farrer, 54 Doughty Street, London WC1N 2LS (GB).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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WO 00/29563 A1

(54) Title: NUCLEIC ACID ISOLATION

(57) Abstract: A method for isolating plasmid DNA from a DNA containing material which comprises plasmid DNA and genomic DNA, comprising extracting the plasmid DNA into a water-immiscible organic solvent capable of supporting plasmid DNA, by mixing the material with the organic solvent, a chaotrope and water under conditions to denature the genomic DNA and recovering the plasmid DNA from the organic phase.

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(71) Applicant (*for all designated States except US*): CAMBRIDGE MOLECULAR TECHNOLOGIES LIMITED [GB/GB]; Unit 3, Cambridge Techno Park, Newmarket Road, Cambridge CB5 8PB (GB).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): BUTT, Neil, James [GB/GB]; 15 Petworth Street, Cambridge CB2 2LY (GB). JOES, Christopher, Peter [GB/GB]; 84 Fowlmere Road, Heydon, Herts SG8 8PU (GB).

(74) Agents: DANIELS, Jeffrey, Nicholas et al.; Page White & Farrer, 54 Doughty Street, London WC1N 2LS (GB).

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Published
With international search report.

(54) Title: NUCLEIC ACID ISOLATION

(57) Abstract

A method for isolating plasmid DNA from a DNA containing material which comprises plasmid DNA and genomic DNA, comprising extracting the plasmid DNA into a water–immiscible organic solvent capable of supporting plasmid DNA, by mixing the material with the organic solvent, a chaotrope and water under conditions to denature the genomic DNA and recovering the plasmid DNA from the organic phase.

FOR THE PURPOSES OF INFORMATION ONLY

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UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

PCT/GB99/03830

WO 00/29563

NUCLEIC ACID ISOLATION

The present invention relates to a method for isolating nucleic acid, and particularly to a method for isolating plasmid DNA from a plasmid DNA-containing material.

Conventional procedures for the purification of nucleic acid, such as DNA, generally require multiple steps including lysis of source material followed by fractionation steps which may involve column chromatography. Where DNA manipulation is to be carried out, small scale DNA preparations are required routinely, often in large quantities for the purpose of screening DNA from the source cells. These processes are time consuming and labour intensive.

Various methods have been proposed in the purification of such DNA, including a precipitation method in EP-A-0376080, an ultrafiltration method in WO-A-87/07645 and EP-A-0517515 and cationic exchange resins in EP-A-0281390 and EP-A-0366438. A simplified method involving a filter, which is automatable, is disclosed in WO-A-95/02049.

Each of these methods suffers from a disadvantage that a series of steps is required and/or special apparatus is required to achieve sufficient purification of the plasmid DNA. A need therefore arises for a much simpler method involving readily-available apparatus and relatively inexpensive reagents. In a known approach for rapid purification of genomic DNA, RNA or protein, a mixture of phenol, chloroform and guanidine is used (Chomczynski, P. and Sacchi, N., 1987 Anal Biochem. 162: 156; Chomczynski, P., 1993 Biotechniques 15: 532) in which the DNA is extracted into an aqueous phase. This method is unsuitable for isolating plasmid DNA. Moreover, the use of phenol and chloroform is undesirable as these are toxic substances.

The present invention aims to overcome the disadvantages of the prior art and to provide a simplified method for isolating plasmid DNA.

Accordingly, the present invention provides a method for isolating plasmid DNA from DNA containing material which comprises plasmid DNA and genomic DNA, comprising:

(i) extracting the plasmid DNA into a water-immiscible organic solvent capable of supporting plasmid DNA, by mixing the material with the organic solvent, a chaotrope and water under conditions to denature the genomic DNA; optionally separating the organic and aqueous phases of step (i); and

(ii) recovering the plasmid DNA from the organic phase.

Accordingly, the present invention provides a "one step" method which is simple to perform and which requires no specialised laboratory apparatus. It is surprisingly found that this method is capable of extracting plasmid DNA to high purity and with particularly low or zero contamination from genomic DNA which might be present in the plasmid DNA-containing material. In a preferred arrangement, the organic solvent is capable of selectively supporting the plasmid DNA with the exclusion of genomic DNA present in the plasmid DNA-containing material.

The method of the present invention may be performed on a small routine laboratory scale working with solution volumes of microlitres or millilitres. Alternatively, the method may be scaled up even to pilot or industrial scale involving volumes of litres or greater.

In extraction step (*i*), the DNA-containing material is mixed with the reagents under conditions to denature the genomic DNA typically whereby the plasmid DNA is partitioned into an organic

phase and the genomic DNA is partitioned into an aqueous phase. Such conditions include basic conditions or elevated temperature. Suitable elevated temperatures are of at least 65°C and more preferably in the range 70 to 95°C for a time sufficient to denature the plasmid DNA such as from about 30s to about 10mins, preferably around five minutes. Incubation times longer than about 10 minutes at elevated temperature should not adversely affect the plasmid DNA but are undesirable for using the organic solvent. In a preferred arrangement, basic conditions are used in which a base is present. The base is typically a hydroxide such as an alkali metal hydroxide, preferably sodium hydroxide. The base is preferably present at a concentration in the range 100mM to 200mM. Incubation time is usually in the range from about 30s to about 10mins, preferably around five minutes. Excessive incubation under basic conditions can damage the plasmid DNA.

Without wishing to be bound by theory, it is thought that differential solubility between plasmid and genomic DNA under denaturing condition may result in plasmid DNA in an undenatured or reversibly denatured state partitioning into the organic phase. In contrast denatured genomic DNA partitions into the aqueous phase.

The organic solvent must be immiscible with the aqueous phase and preferably comprises an alcohol which may be aliphatic or aromatic and which may be linear or branched chain. The alcohol is preferably a C_3 to C_6 alcohol, more preferably a C_4 to C_6 alcohol and most preferably comprises a butanol such as N-butanol.

The chaotrope may be any normally-recognised chaotrope and is preferably selected from guanidine hydrochloride, guanidine thiocyanate, sodium perchlorate and mixtures thereof. A preferred chaotrope is guanidine hydrochloride. Typically, the

chaotrope is present at a concentration in the range 0.7M to 1.2M, based on the combination of organic solvents, chaotrope water. The concentration of the chaotrope is preferably about 0.9M.

The amount of organic solvent is typically in the range from 20 to 70% based on the volume of the combination of organic solvent, chaotrope or water and is preferably in the range from 35 to 50%, more preferably around 42%.

The exact organic solvent, chaotrope, base and amounts thereof are readily determinable by routine experimentation. Each of these reagents may be mixed with the DNA-containing material in any order or may be premixed prior to addition to the plasmid-containing material. In a convenient arrangement, the organic solvent, chaotrope, base and water are combined to form an extraction mixture. In this arrangement, the extraction step (*i*) comprises mixing the extraction mixture with the DNA-containing material.

At laboratory scale, the step (*ii*) of separating the organic and aqueous phases may be conveniently carried out by allowing the phases to separate or encouraging separation on the basis of density by a short spin in a microcentrifuge. Typically, either the organic or aqueous phase is removed from the other prior to recovery step (*iii*). For example, the organic phase containing the plasmid DNA may be transferred from one container to another by pipette prior to recovery. On a larger scale, removal of one phase from the other could be performed by any conventional method including pumping or running off by gravity one of the two phases.

In one arrangement, recovery step (*iii*) includes precipitation of the plasmid DNA from the organic solvent. For example, the DNA-containing organic phase may be mixed with a precipitating

agent that can precipitate the plasmid DNA from the organic solvent and the precipitated plasmid DNA is separated from the solvent. The precipitated plasmid DNA may also be washed in a washing step. The precipitating agent may comprise an alcohol such as ethanol and may further comprise an acetate salt such as sodium acetate.

The DNA-containing material may comprise any known DNA-containing material such as a bacterial culture which may be lysed or unlysed.

In a further aspect, the present invention provides an extraction mixture for selectively extracting plasmid DNA from a DNA-containing material, which extraction mixture comprises a water-immiscible organic solvent capable of supporting plasmid DNA, a chaotrope and water. The extraction mixture preferably further comprises a base.

The organic solvent, chaotrope, base and amounts thereof are typically those described above.

The present invention will now be described in further detail, by way of example only, with reference to the following Examples.

Example 1

General procedure

Bacterial culture (*E coli* containing pBluescript; 0.5ml) was spun down in an eppendorf tube using a microcentrifuge and the supernatant was discarded. The pellet was resuspended in TE buffer (tris[hydroxymethyl]aminomethane hydrochloride 10mM, EDTA 1mM; pH8.0; 200μl) to form a resuspended pellet containing both genomic and plasmid DNA. An extraction mixture was selected according to the Table below, mixed very well and 0.5ml thereof was added to the resuspended pellet and gently mixed. The eppendorf containing the mixture was spun in a microcentrifuge

for 30 seconds to yield two phases; an upper organic phase and a lower aqueous phase. The organic phase was removed carefully to a fresh eppendorf tube avoiding any contaminating debris. Following measurement of the volume of the removed organic phase, sodium acetate (0.1vols; 3M) and ethanol were added (2vols) to precipitate the plasmid DNA. The eppendorf was spun in a microcentrifuge for 20 minutes and the ethanol supernatant removed. The pellet was rinsed with fresh ethanol (70%; 200μl) and spun for 5 minutes. The ethanol was removed and the pellet dried and resuspended in water (20μl). The resultant plasmid-containing DNA solution could then be assayed by visualisation on an agarose gel and the amount of DNA determined quantitatively by spectrophotometry or by fluorescence.

Table of Extraction Mixtures Tested

CHAOTROPE	NaOH	SOLVENT	PLASMID DNA RECOVERY
GuSCN 0.9M	150mM	N-Butanol 42%	Poor
GuSCN 0.9M	90mM	N-Butanol 42%	Poor
GuSCN 0.9M	200mM	N-Butanol 42%	Poor
GuSCN 0.9M	90mM	N-Butanol 20%	Poor
GuSCN 0.9M	150mM	N-Butanol 20%	Poor
GuSCN 0.9M	200mM	N-Butanol 20%	Good
GuSCN 0.9M	90mM	N-Butanol 70%	No
GuSCN 0.9M	150mM	N-Butanol 70%	No
GuSCN 0.9M	200mM	N-Butanol 70%	No
GuHCl 0.9M	90mM	N-Butanol 42%	Good
GuHCl 0.9M	150mM	N-Butanol 42%	Good
GuHCl 0.9M	200mM	N-Butanol 42%	Good
GuHCl 0.9M	90mM	N-Butanol 20%	OK
GuHCl 0.9M	150mM	N-Butanol 20%	OK
GuHCl 0.9M	200mM	N-Butanol 20%	Poor
GuHCl 0.9M	90mM	N-Butanol 70%	OK
GuHCl 0.9M	150mM	N-Butanol 70%	OK
GuHCl 0.9M	200mM	N-Butanol 70%	Poor
GuHCl 0.9M	90mM	2 methyl propanol 20%	Poor
GuHCl 0.9M	150mM	2 methyl propanol 20%	Poor
GuHCl 0.9M	200mM	2 methyl propanol 20%	Poor
GuHCl 0.9M	90mM	2 methyl propanol 70%	No
GuHCl 0.9M	150mM	2 methyl propanol 70%	No
GuHCl 0.9M	200mM	2 methyl propanol 70%	No
GuHCl 0.9M	90mM	2 methyl propanol 42%	Poor
GuHCl 0.9M	150mM	2 methyl propanol 42%	OK
GuHCl 0.9M	200mM	2 methyl propanol 42%	OK

GuHCl	0.9M	90mM	Butan-2-ol 42%	Poor
GuHCl	0.9M	150mM	Butan-2-ol 42%	OK
GuHCl	0.9M	200mM	Butan-2-ol 42%	Good
GuHCl	0.9M	90mM	Butan-2-ol 20%	Poor
GuHCl	0.9M	150mM	Butan-2-ol 20%	Poor
GuHCl	0.9M	200mM	Butan-2-ol 20%	Poor
Na Perchlorate	0.9M	90mM	N-Butanol 42%	Poor
Na Perchlorate	0.9M	150mM	N-Butanol 42%	Poor
Na Perchlorate	0.9M	200mM	N-Butanol 42%	Poor
Na Perchlorate	0.9M	90mM	N-Butanol 70%	Poor
Na Perchlorate	0.9M	150mM	N-Butanol 70%	Poor
Na Perchlorate	0.9M	200mM	N-Butanol 70%	Poor
Na Perchlorate	0.9M	90mM	N-Butanol 20%	Poor
Na Perchlorate	0.9M	150mM	N-Butanol 20%	Poor
Na Perchlorate	0.9M	200mM	N-Butanol 20%	Poor
Na Perchlorate	0.9M	200mM	2 methyl propanol 20%	OK
Na Perchlorate	0.9M	90mM	2 methyl propanol 70%	OK
Na Perchlorate	0.9M	150mM	2 methyl propanol 70%	Poor
Na Perchlorate	0.9M	200mM	2 methyl propanol 70%	Poor
Na Perchlorate	0.9M	90mM	Butan-2-ol 42%	Poor
Na Perchlorate	0.9M	150mM	Butan-2-ol 42%	Poor
Na Perchlorate	0.9M	200mM	Butan-2-ol 42%	OK
Na Perchlorate	0.9M	90mM	Butan-2-ol 20%	No
Na Perchlorate	0.9M	150mM	Butan-2-ol 20%	No
Na Perchlorate	0.9M	200mM	Butan-2-ol 20%	No

Good	Approximately 1μg DNA recovery
OK	Approximately 200ng DNA recovery
Poor	Just visible on agarose gel electrophoresis

It may be concluded from these results that each recognised chaotrope works and that the guanidine hydrochloride is preferred over the guanidine thiocyanate which is, in turn, preferred over sodium perchlorate in terms of DNA recovery. As to solvents, butanol was found to work best whereas pentanol gave only poor DNA recovery. Ethanol and isopropanol were found not to be water-immiscible. Of the butanols, N-butanol was found to be better than either butan-2-ol or 2 methyl propanol.

Whilst TE was used as the resuspension buffer in the procedure, water could also be used, as well as other resuspension buffers.

Example 2

General procedure for extraction using heat instead of alkaline pH

Bacterial culture (*E coli* containing pBluescript; 0.5ml) was spun down in an eppendorf tube using a microcentrifuge and the supernatant was discarded. The pellet was resuspended in TE buffer (tris[hydroxymethyl]aminomethane hydrochloride 10mM, EDTA 1mM; pH8.0; 200μl) to form a resuspended pellet containing both genomic and plasmid DNA. An extraction mixture was selected according to the Table below, mixed very well and 0.5ml was added to resuspended pellet and gently mixed. The eppendorf tube was then placed in a hot water bath at a temperature in the range 70 to 95°C for five minutes and the contents frequently mixed. Care was taken with the lid of the eppendorf tube because of solvent expansion in the tube. The tube was then rapidly cooled on ice for three minutes, which had the effect of separating the plasmid and genomic DNA. The eppendorf containing the mixture was spun in a microcentrifuge for 30 seconds to yield two phases; an upper organic phase and a lower aqueous phase. The organic phase was removed carefully to a fresh eppendorf tube avoiding any contaminating debris. Following measurement of the volume of the removed organic phase, sodium acetate (0.1vols; 3M) and ethanol were added (2vols) to precipitate the plasmid DNA. The eppendorf was spun in a microcentrifuge for 20 minutes and the ethanol supernatant removed. The pellet was rinsed with fresh ethanol (70%; 200μl) and spun for 5 minutes. The ethanol was removed and the pellet dried and resuspended in water (20μl). The resultant plasmid-containing DNA solution could then be assayed by visualisation on an agarose gel and the amount of DNA determined quantitatively by spectrophotometry or fluorescence.

Results comparable to those of Example 1 were obtained although yields were slightly lower and minor contamination with genomic DNA was observed.

CLAIMS:

1. A method for isolating plasmid DNA from a DNA containing material which comprises plasmid DNA and genomic DNA, comprising:

(i) extracting the plasmid DNA into a water-immiscible organic solvent capable of supporting plasmid DNA, by mixing the material with the organic solvent, a chaotrope and water under conditions to denature the genomic DNA; and

(ii) recovering the plasmid DNA from the organic phase.

2. A method as claimed in claim 1, wherein the organic solvent is capable of selectively supporting the plasmid DNA to the exclusion of denatured genomic DNA.

3. A method as claimed in claim 1 or claim 2, wherein the conditions to denature the plasmid DNA comprise basic conditions or a temperature of at least 65°C.

4. The method as claimed in claim 3, wherein the conditions to denature the plasmid DNA comprise basic conditions in which a base is present.

5. A method as claimed in claim 4, wherein the organic solvent comprises a C_3 to C_6 alcohol.

6. A method as claimed in claim 5, wherein the C_3 to C_6 alcohol comprises butanol.

7. A method as claimed in claim 6, wherein the butanol comprises n-butanol.

8. A method as claimed in any one of claims 4 to 7, wherein the chaotrope is selected from the group consisting of guanidine

hydrochloride, guanidine thiocyanate, sodium perchlorate and mixtures thereof.

9. A method as claimed in claim 8, wherein the chaotrope comprises guanidine hydrochloride.

10. A method as claimed in any one of claims 4 to 9, wherein the base comprises a hydroxide.

11. A method as claimed in claim 10, wherein the hydroxide comprises sodium hydroxide.

12. A method as claimed in any one of claims 4 to 11, wherein the organic solvent, the chaotrope, the base and the water are combined to form an extraction mixture, and extraction step (i) comprises mixing the extraction mixture with the plasmid DNA-containing material.

13. A method as claimed in any one of claims 4 to 12, wherein the amount of organic solvent is in the range from 20 to 70% based on the volume of the combination of organic solvent, chaotrope and water.

14. A method as claimed in claim 13, wherein the amount of the organic solvent is in the range from 35 to 50%.

15. A method as claimed in claim 14, wherein the amount of the organic solvent is about 42%.

16. A method as claimed in any one of claims 4 to 15, wherein the chaotrope is present at a concentration of from 0.7M to 1.2M based on the combination of organic solvent, chaotrope and water.

17. A method as claimed in claim 16, wherein the concentration of the chaotrope is about 0.9M.

18. A method as claimed in any one of claims 4 to 17, wherein the recovery step (ii) comprises mixing the DNA-containing organic phase with a precipitating agent that can precipitate the plasmid DNA from the organic solvent, and separating the precipitated plasmid DNA from the solvent.

19. A method as claimed in claim 18, wherein the recovery step (ii) further comprises a washing step in which the precipitated plasmid DNA is washed.

20. A method as claimed in claim 18 or claim 19, wherein the precipitating agent comprises an alcohol.

21. A method as claimed in claim 20, wherein the alcohol comprises ethanol.

22. A method as claimed in any one of claims 18 to 21, wherein the precipitating agent further comprises an acetate salt.

23. A method as claimed in claim 22, wherein the acetate salt comprises sodium acetate.

24. A method as claimed in any one of the preceding claims, which further comprises a step of separating the organic and aqueous phases of step (i) prior to recovering the plasmid DNA.

25. A method as claimed in claim 24, wherein the step of separating the organic and aqueous phases further comprises centrifugation of the mixture formed in step (i) to facilitate separation of the mixture into the organic and aqueous phases.

26. A method as claimed in any one of the preceding claims, wherein the DNA-containing material comprises a lysed or unlysed bacterial culture.

27. An extraction mixture for selectively extracting plasmid DNA from a DNA-containing material, which extraction mixture comprises a water-immiscible organic solvent capable of supporting plasmid DNA, a chaotrope and water.

28. An extraction mixture as claimed in claim 27, which further comprises a base.

29. An extraction mixture as claimed in claim 28, wherein the base comprises a hydroxide.

30. An extraction mixture as claimed in claim 29, wherein the hydroxide comprises sodium hydroxide.

31. An extraction mixture as claimed in any one of claims 27 to 30, wherein the organic solvent is capable of selectively supporting plasmid DNA to the exclusion of genomic DNA.

32. An extraction mixture as claimed in any one of claims 27 to 31, wherein the organic solvent comprises a C_3 to C_6 alcohol.

33. An extraction mixture as claimed in any one of claims 27 to 32, wherein the organic solvent comprises butanol.

34. An extraction mixture as claimed in claim 33, wherein the butanol comprises n-butanol.

35. An extraction mixture as claimed in any one of claims 27 to 33, wherein the organic solvent constitutes from 20 to 70% based on the volume of the extraction mixture.

36. An extraction mixture as claimed in claim 35, wherein the organic solvent constitutes from 35 to 50 % of the extraction mixture.

37. An extraction mixture as claimed in claim 36, wherein the organic solvent constitutes about 42% of the extraction mixture.

38. An extraction mixture as claimed in any one of claims 27 to 37, wherein the chaotrope is selected from the group consisting of guanidine hydrochloride, guanidine thiocyanate, sodium perchlorate and mixtures thereof.

39. An extraction mixture as claimed in claim 38, wherein the chaotrope comprises guanidine hydrochloride.

40. An extraction mixture as claimed in any one of claims 27 to 39, wherein the concentration of chaotrope in the extraction mixture is from 0.7M to 1.2M.

41. An extraction mixture as claimed in claim 40, wherein the concentration of the chaotrope in the extraction mixture is about 0.9M.

ATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
100794/JD/JE		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 99/03830	17/11/1999	17/11/1998
Applicant		
CAMBRIDGE MOLECULAR TECHNOLOGIES LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 2 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[ ] the text is approved as submitted by the applicant.

[X] the text has been established by this Authority to read as follows:

NUCLEIC ACID ISOLATION

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. –

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/03830

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 637 687 A (WIGGINS JAMES C) 10 June 1997 (1997-06-10) claim 1 ---	1-41
A	US 5 643 767 A (FISCHETTI VINCENT A ET AL) 1 July 1997 (1997-07-01) claim 35 -----	1-41

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 January 2000	01/02/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mata Vicente, T.

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 99/03830

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5637687 A	10-06-1997	NONE	
US 5643767 A	01-07-1997	EP 0755401 A	29-01-1997
		JP 10501685 T	17-02-1998
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